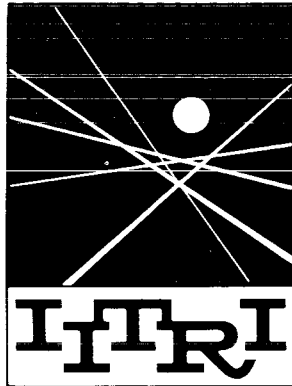


(NASA CR 50934)

16p.

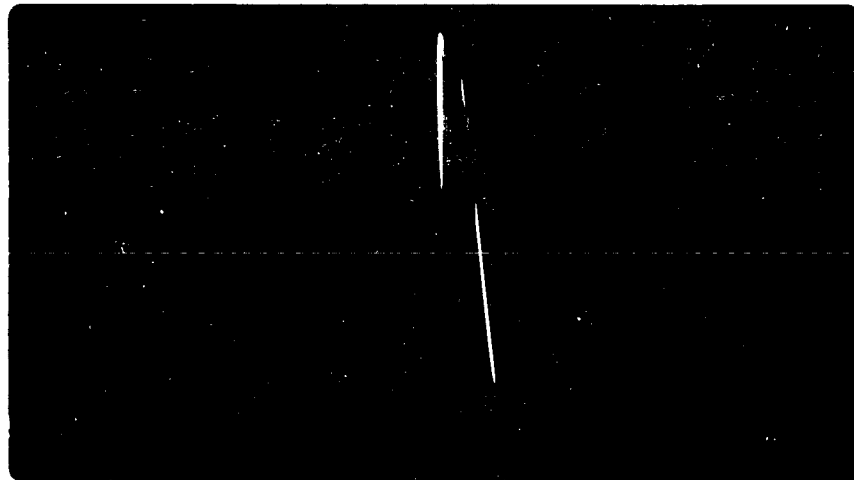
N 64 22758

Oct 16



IIT RESEARCH INSTITUTE

formerly Armour Research Foundation of Illinois Institute of Technology



Technology Center

Chicago 16, Illinois

OTS PRICE

XEROX

\$

MICROFILM

\$

Report No. IITRI 3194-10
(Quarterly Status Report)

LIFE IN EXTRATERRESTRIAL ENVIRONMENTS

National Aeronautics
and Space Administration
Washington, D.C.

~~Available to NASA Offices and~~
~~NASA Contractors~~

IIT RESEARCH INSTITUTE

(NASA CA - ;

cover
for
availability
report

Report No. IITRI-C194-10
② Quarterly Status Report

① LIFE IN EXTRATERRESTRIAL ENVIRONMENTS

③ May 15 to August 15, 1963

National Aeronautics and Space Administration

(NASA Contract No. NASr-22)
IITRI Project C194

I. INTRODUCTION

Charles A. HAGEN [1963]

Gregor

Numerous soil samples have been collected from the California Deserts*, tundra areas (12,200 ft) of the Rocky Mountain National Park, and the desert of White Sands National Monument. The indigenous microorganisms have been isolated and are being studied in the simulated Martian environment.

From the previously reported (IITRI Report No. C194-9) California desert soil samples, 253 bacterial cultures were obtained. Subsequent tests have revealed that 88 cultures were facultative anaerobes. These cultures are being examined for survival in a simulated Martian environment for 28 days.

Survival after 112 days in the simulated Martian environment of a Micrococcus isolated from California desert soil is recorded. Biochemical tests and morphological characteristics identified this organism as Sarcina aurantiaca.

* Desert soil samples supplied by Dr. R. E. Cameron, Jet Propulsion Laboratory, California Institute of Technology, California.

IIT RESEARCH INSTITUTE, Chicago, Ill.

424 7300 - 1 -

A Bacillus isolated from California desert soil and identified as Bacillus cereus var. mycoides has been examined for survival after 28 days in the simulated Martian environment.

Preliminary experiments on intestinal bacteria present in rat feces indicated that less than 10% of the coliform-type bacteria survive the inoculation and flushing procedures used to establish the simulated Martian environment.

Experimental work has been initiated to study the survival in the simulated Martian environment of a chemolithotrophic bacterium, Desulfovibrio desulfuricans, and two photosynthetic, purple bacteria, Rhodopseudomonas spheroides and Rhodospirillum rubrum.

Studies have been initiated to examine the survival and growth characteristics of tundra and desert plants. In addition to Martian survival experiments, these plants will be examined for their ability to withstand varying intensities and wavelengths of light, their minimum moisture and nutrient requirements, and tolerance to various atmospheric compositions and temperatures.

II. EXPERIMENTAL PROCEDURES

The simulated Martian atmosphere described in Report 3194-5 was used. The methods of growing, harvesting, inoculating, and sampling the Mars tubes and the types of media were described in Reports No. 3194-2, -3, -4, and -7.

The screening program involving 253 isolates from five California desert soils was conducted in the following manner. The organisms were streaked on the surface of liver veal agar (Difco) and incubated at 24°C for 7 days in Case anaerobars (Case Laboratories, Chicago, Illinois) containing an atmosphere of 95% nitrogen and 5% carbon dioxide. The 88 cultures that grew were classified as facultative anaerobes. These cultures are being examined for survival after 28 days in the Martian environment by pour plate method with trypticase soy agar (Baltimore Biological Laboratories).

The biochemical tests were performed in accordance with standard microbiological methods.¹⁻⁴

Suspensions of monkey rat, and guinea pig feces were prepared by emulsifying 0.5 g of feces in 3 ml of 0.1% peptone water to give bacterial counts of 10^4 to 10^6 cells/0.01 ml. The suspensions were inoculated in sterile desert soil No. 62. Trypticase soy agar was used for total counts and MaConkey's agar (Difco) was used for coliform counts.

¹Conn, H. J., "Manual of Microbiological Methods," Williams and Wilkins Co., Baltimore, 1957.

²Skerman, V. B. D., "A Guide to the Identification of the Genera of Bacteria," William and Wilkins Co., Baltimore, 1959.

³Breed, R. S., Murray, E. G. D., and Smith, N. R., "Bergey's Manual of Determinative Bacteriology," William and Wilkins Co., Baltimore, 1957.

⁴Smith, N. R., Gordon, R. E., and Clark, F. E., "Aerobic Spore-forming Bacteria," U.S. Department of Agriculture Monograph No. 16, U.S. Government Printing Office, Washington, D.C., 1952.

The cultures of Desulfovibrio desulfuricans ATCC 7757, Rhodopseudomonas spheroides ATCC 11167, and Rhodospirillum rubrum ATCC 9791 were obtained from the American Type Culture Collection. D. desulfuricans was cultured on Starkey's medium², R. spheroides, according to techniques and media suggested by Skerman², and R. rubrum in fluid thioglycollate medium (Oxoid). All cultures were incubated at 27°C

III. RESULTS AND DISCUSSION

Table 1 lists the viable counts from the screening experiments of 253 desert soil isolates. In addition to the 12 cultures listed in Table 1, 11 other cultures are being tested.

Figure 1 shows the survival of the Sarcina isolated from California desert soil No. 62 in the simulated Martian environment after 112 days. The percent moisture of both the Earth control and Martian experimental groups was 0.18%. A slight decrease in viable cells occurred over the 112-day exposure period. The Earth control group had 44×10^6 cells/g initially, and at 112 days 21×10^6 cells/g were present; the Martian experimental group had 30×10^6 cells/g initially, and at 112 days 25×10^6 were present. The viable cell count should decrease with extended exposure periods as a result of starvation. However, the low moisture content of the system could enhance the resistance as usually observed in lyophilized cultures.

Table 1

SURVIVAL AFTER 28 DAYS IN MARTIAN ENVIRONMENT OF
MICROORGANISMS ISOLATED FROM CALIFORNIA DESERT SOILS

Culture No.	Source**	Organism	Number of Bacteria/g*			
			0 Day		28 Days	
			Total Count	Spore Count	Total Count	Spore Count
55	68	Bacillus	25×10^5	61×10^4	44×10^4	91×10^4
8	51	Bacillus	63×10^5	10×10^4	11×10^4	17×10^4
12	51	Bacillus	26×10^5	48×10^4	81×10^4	98×10^4
33	62	Bacillus	27×10^5	62×10^4	77×10^4	83×10^4
37	62	Bacillus	20×10^5	4×10^4	44×10^4	29×10^4
54	68	Bacillus	46×10^5	46×10^4	17×10^5	22×10^5
34	62	Coccus	25×10^3		$< 10^3$	
56	68	Bacillus	30×10^5	23×10^5	17×10^5	97×10^4
57	68	Bacillus	15×10^5	21×10^5	30×10^4	68×10^4
60	70	Bacillus	28×10^4	36×10^4	44×10^4	34×10^4
61	70	Bacillus	36×10^5	34×10^5	31×10^5	23×10^5
68	70	Bacillus	79×10^5	70×10^5	67×10^5	11×10^5

* Desert soil No. 62 was used as the substrate in the screening experiments.

**Desert Soils: Supplied by Dr. R. E. Cameron of Jet Propulsion Laboratories, California Institute of Technology.

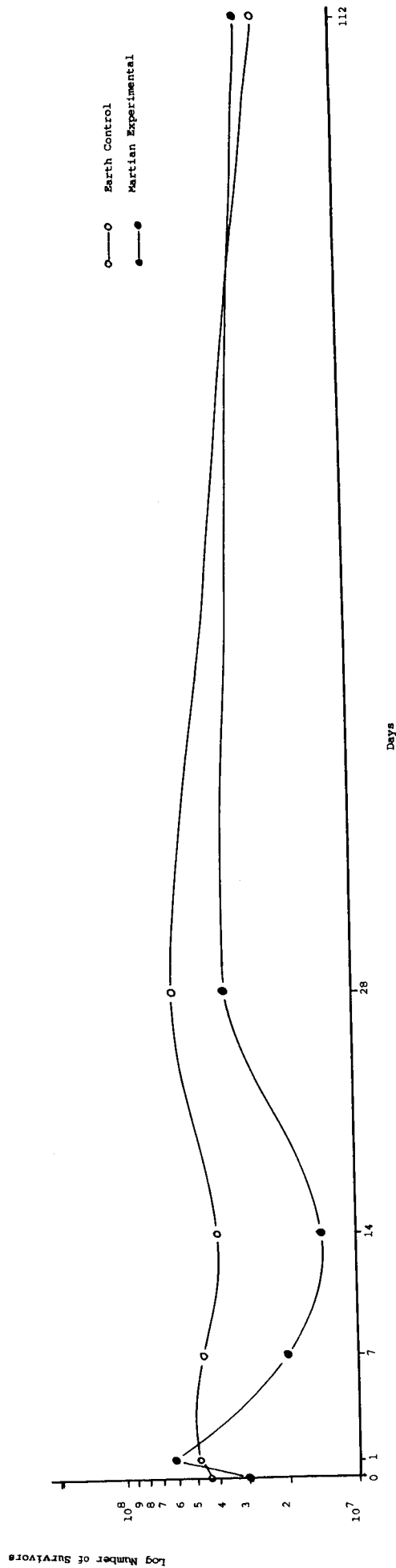
50 - Sand

62 - Sandy Loam

68 - Clay

70 - Sand

Figure 1
SURVIVAL IN MARTIAN ENVIRONMENT OF A MICROCOCCLUS IN MARTIAN SOIL



The survival of Sarcina in sterile California desert soil No. 62, in the Martian environment for 112 days is shown in Figure 2. The percent moisture of the Earth control group was 0.53% and that of the Martian experimental group 0.51%. The number of cells surviving the 112-day exposure period were similar in the two groups: 132×10^6 cells/g and 127×10^6 cells/g the Earth and Martian experimental groups, respectively. It is significant that this non-sporeforming microorganism demonstrated a high survivability in the simulated Martian environment.

The morphological characteristics and results of biochemical tests of this organism are given in Table 2. The organism was identified as closely resembling Sarcina aurantiaca. The nitrate reduction and the indole tests were prepared in triplicate and examined after 24 and 48 hr and 7 days. Citrate utilization was regarded as negative when after transferring from citrate to citrate medium no turbidity was observed after 7 days. This procedure rules out the possibility of turbidity being produced in the citrate medium as a result of carryover of nutrients from the inoculating broth suspensions. These tests were performed at 25 and 37°C, and no variation in results occurred.

Little information regarding the heat sensitivity of vegetative cells of Sarcina is available apart from the observations by Gibson⁵ who reported that vegetative cells of S. ureae withstand

⁵Gibson, T., Arch. Mikrobiol. 6, 73-78, 1935.

Figure 2
SURVIVAL IN MARTIAN ENVIRONMENT OF A MICROCOCOCCUS IN DESERT SOIL

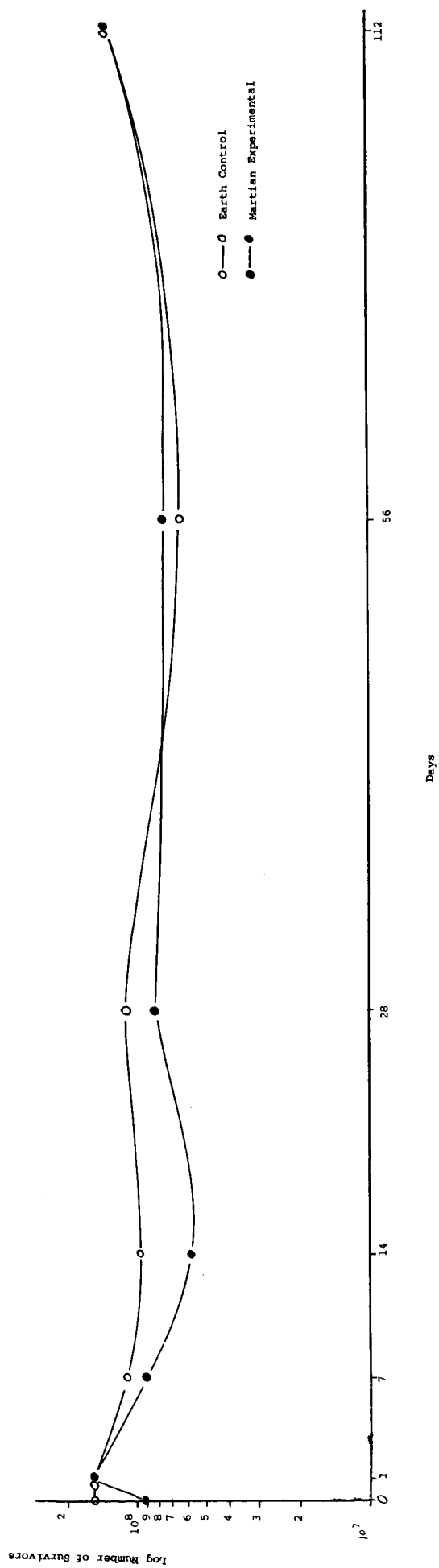


Table 2

MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS
OF A MICROCOCCUS ISOLATED FROM DESERT SOIL

Vegetative cells:	0.9 to 1.1 μ in diameter; singly, in pairs, and in clumps; non-motile; Gram-positive.
Spores:	Not present.
Agar colonies:	Circular, smooth, orange, glistening, raised to convex.
Agar slant:	Abundant, opaque, orange, filiform, smooth, moist.
Broth:	Light turbidity, no pellicle, orange slimy sediment.
Litmus milk:	No change.
Reduction of nitrate to nitrite:	Negative.
Production of indole:	Negative.
Utilization of $\text{NH}_4\text{H}_2\text{PO}_4$:	Positive.
Utilization of citrate:	Negative.
Hydrolysis of starch:	Positive.
Hydrolysis of gelatin:	Positive.
Fermentation tests:	No acid from glucose, glycerol, lactose, maltose, raffinose, or sucrose.
Catalase present:	Positive.
Urease present:	Negative.
Heat resistance:	Withstood 60°C for 10 min, but not 80°C for 10 min.
Oxygen requirement:	Obligate aerobe.

exposure to 75°C for 15 min and by MacDonald and MacDonald⁶ who reported that vegetative cells of S. ureae and S. flava withstand 60°C for 10 min. The value of MacDonald and MacDonald is similar to ours. The desert soil isolate resembles Group II Sarcina as described by MacDonald and MacDonald: non-motile, non-sporeforming Sarcina that hydrolyzes starch and gelatin, catalase positive and urease negative, and do not produce acid from carbohydrates. Members of this group are S. subflava, S. flava, S. aurantiaca, and S. lutea.

The Bacillus isolated from California desert soil No. 68 has completed initial screening tests in the simulated Martian environment (Table 1, culture No. 54). After 28 days 37% of the cells were recovered as total count while the spore count increased 4.9 times. This organism is currently being tested for survival in the simulated Martian environment for periods greater than 28 days. Viable cell counts are being determined as total and spore counts grown under aerobic and anaerobic conditions. Any change in the organism's oxygen requirement occurring as a result of prolonged exposure will be determined. Table 3 lists the morphological characteristics and results of biochemical tests of this organism. All tests were continued for 7 days. The same precautions for detection of indole production, reduction of nitrates, and citrate utilization described previously were observed. Temperature of incubation was 35°C.

⁶ MacDonald, R. E. and MacDonald, S. W., Can. J. Microbiol. 8, 795-808, 1962.

Table 3

MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS
OF BACILLUS ISOLATED FROM DESERT SOIL

Vegetative cells:	3.3 by 0.8 μ ; singly, in pairs, and in short chains; ends rounded; stained protoplasm granular or foamy; Gram-positive; non-motile.
Spores:	Oval, 1.8 x 1.0 μ long; central or paracentral.
Sporangia:	Not appreciably swollen.
Nutrient agar colonies:	Large, rough, flat, irregular with whiplike outgrowths, white to off-white.
Nutrient agar slant:	Abundant, rough, opaque, white to off-white, adherent, spreading with irregular edge.
Broth:	Light turbidity with flocculent sediment, soft ring pellicle.
Reduction of nitrate to nitrite:	Positive.
Production of indole:	Negative.
Production of acetylmethylcarbinol:	Positive.
Utilization of KNO_3 :	Negative.
Utilization of Citrate:	Positive
Hydrolysis of starch:	Positive.
Hydrolysis of gelatin:	Positive.
Hydrolysis of casein:	Positive.
Lecithinase reaction:	Positive.

Table 3 (cont)

MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS
OF A BACILLUS ISOLATED FROM DESERT SOIL

Fermentation tests:	Acid without gas from glucose, maltose, mannitol, and sucrose; no gas from glycerol, lactose, raffinose, or xylose.
Catalase present:	Positive.
Urease present:	Negative.
Oxygen requirement:	Facultative anaerobe.

The organism was classified as Bacillus cereus var mycoides.

Table 4 shows the effect of inoculating and flushing procedures on the survival of intestinal bacteria in sterile soils which were inoculated with rat, monkey, and guinea pig feces. Preliminary tests indicated at least 10% of the bacterial population survived. The number of coliform bacteria should be sufficiently high in order to study the effects of the simulated Martian environment on the survival of the various bacterial types present in the animal's intestinal tracts.

IV. SUMMARY

Eighty-eight cultures of facultative anaerobic bacteria were obtained from 253 isolates from various California desert soils. The cultures are being screened for survival in the simulated Martian environment.

A non-sporeforming organism identified as S. aurantiaca demonstrated a high resistance to the simulated Martian environment, after 112 days exposure.

Initial screening tests indicated good survival of a Bacillus isolated from California desert soil No. 68; 37% of the cells were recovered as total count and the spore count increased 4.9 times after 28 day exposure to the simulated Martian environment. This organism was identified as Bacillus cereus var mycoides from results of biochemical tests and morphological characteristics. Further studies are planned with this microorganism.

IIT RESEARCH INSTITUTE

Table 4

EFFECT OF INOCULATING AND FLUSHING PROCEDURES ON
SURVIVAL OF INTESTINAL BACTERIA IN SOILS INOCULATED WITH
MONKEY, RAT, AND GUINEA PIG FECES

Animal Source	Number of Bacteria/g			
	Before Inoculation and Flushing		After Inoculation and Flushing	
	Total Count	Coliform Count	Total Count	Coliform Count
Monkey	58×10^3	$<10^2$	35×10^3	$<10^2$
Rat	65×10^4	20×10^3	72×10^3	$<10^2$
Guinea pig	63×10^3	$<10^2$	60×10^2	$<10^2$

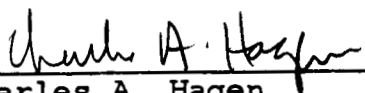
Suspensions of monkey, rat, and guinea pig feces inoculated into sterile desert soil were subjected to flushing procedures normally used to establish the simulated Martian environment. Experimentation is in progress to establish the survivability of organisms from metabolic waste material in the simulated Martian environment. Also studies were initiated with selected tundra and desert plants to determine their survival, in the simulated Martian environment.

V. RECORDS AND PERSONNEL

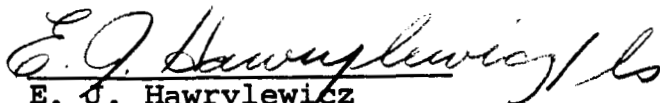
The experimental data are recorded in Logbooks C13027, C13248, C13740, and C13783. Technical assistance was given by John Rush.

Respectfully submitted,

IIT RESEARCH INSTITUTE


Charles A. Hagen
Associate Bacteriologist
Life Sciences Research

Approved by:


E. J. Hawrylewicz
Manager
Life Sciences Research

CAH/lr

IIT RESEARCH INSTITUTE

Distribution of NASA-22 doc (ARR)

DISTRIBUTION LIST

This report is being distributed as follows:

Copy No. _____

<u>Copy No.</u>	<u>Recipient</u>
1 - 25	Office of Grants and Research Contracts Office of Space Sciences National Aeronautics and Space Administration Washington 25, D.C. Attention: SC
26	IIT Research Institute Division L Files
27	IIT Research Institute Editors, G. S. Gordon, Main Files
28	IIT Research Institute K. W. Miller, Report Library
29	Dr. Irving Davis Major, USAF, MSC European Office Office of Aerospace Research Shell Building, 45 Cantersteen Brussels, Belgium
30	Dr. M. H. Halpern RCA, Defense Electronic Products Camden 2, New Jersey
31	Dr. C. S. Pittendrigh Department of Biology Princeton University P. O. Box 704 Princeton, New Jersey
32	Dr. Allan Brown Department of Botany University of Minnesota Minneapolis, Minnesota

<u>Copy No.</u>	<u>Recipient</u>
33	Dr. E. C. Polard Visiting Professor of Biophysics College of Chemistry and Physics Pennsylvania State Univeristy University Park, Pennsylvania
34	Dr. Norman Horowitz Biology Department California Institute of Technology Pasadena, California
35	Dr. Kirby-Smith Biology Division Oak Ridge National Laboratory P. O. Box Y Oak Ridge, Tennessee
36	Dr. Sidney Fox Florida State University Tallahassee, Florida
37	Dr. Melvin Calvin Space Sciences Laboratory University of California Berkeley 4, California
38	Dr. Carl Sagan Department of Astronomy University of California Berkeley, California
39	Dr. Dale Smith Office of Space Sciences National Aeronautics and Space Administration 400 Maryland Avenue, S. W. Washington 25, D. C.